American Journal of Hematology 57:131–138 (1998)

Immunologic Effects of Anti-D (WinRho-SD) in Children With Immune Thrombocytopenic Purpura

Sherri A. Zimmerman,[1]* Frank J. Malinoski,[2] and Russell E. Ware[1]

[1]Department of Pediatrics, Division of Hematology/Oncology, Duke University Medical Center, Durham, North Carolina
[2]NABI, Rockville, Maryland

Intravenous immunoglobulin (IVIG) is an effective treatment for immune thrombocytopenic purpura (ITP) that induces transient blockade of the reticuloendothelial system (RES) with additional effects including alteration of T lymphocyte subsets and suppression of in vitro T lymphocyte proliferation. As anti-D also is an effective treatment for ITP, we investigated its in vitro and in vivo immunologic effects. The in vitro effects of various agents used in ITP therapy were compared using T lymphocyte proliferation assays. Anti-D caused significantly less inhibition than IVIG or dexamethasone, but non-specific protein was as suppressive as IVIG. Six children with chronic ITP were studied following anti-D administration. Patients received a single dose of anti-D (WinRho-SD, 50 µg/kg IV over 5 min) and were studied on day 0, day 7, and 1 month later. Anti-D did not affect T lymphocyte subsets including the T cell receptor variable beta repertoire, in vitro T lymphocyte proliferation to mitogens, recall antigens, or interleukin-2, in vitro IgG synthesis induced by pokeweed mitogen, or T lymphocyte cytokine mRNA levels. We conclude that anti-D has no demonstrable in vitro or in vivo effects on lymphocyte enumeration or function, and therefore likely is effective in the treatment of ITP primarily through RES blockade. Am. J. Hematol. 57:131–138, 1998. © 1998 Wiley-Liss, Inc.

Key words: anti-D; immune thrombocytopenic purpura (ITP); T lymphocytes

INTRODUCTION

Immune thrombocytopenic purpura (ITP) is a hematologic disorder characterized by anti-platelet antibodies that lead to immune-mediated platelet destruction by the reticuloendothelial system (RES). Most children who develop ITP have an acute clinical course lasting less than 6 months, while 20% of children develop a chronic clinical course [1]. Depending on the degree of thrombocytopenia and clinical bleeding manifestations, therapeutic options in children with ITP include observation alone, periodic treatment with corticosteroids, intravenous immunoglobulin (IVIG) or anti-D, chronic administration of immunosuppressive agents, or splenectomy [2].

Based on initial observations by Imbach et al. [3,4] that children with congenital or acquired hypogammaglobulinemia and thrombocytopenia had improvement in their platelet count following IVIG administration, IVIG was shown to be effective therapy for ITP in children and adults [5–8]. The mechanism of action of IVIG in ITP has been extensively studied, and its primary effect is to induce RES blockade by competitive inhibition for Fc receptors [4,5] and by decreasing the numbers and affinity of Fc receptors for IgG-coated platelets [9,10]. IVIG has additional effects on the immune system, including alteration of T lymphocyte subsets [11–13], suppression of in vitro T lymphocyte proliferation [14–16], alterations of B cell function [11,12,17], and effects on platelet autoantibody production [11,18,19].

Anti-D is a polyclonal antiserum against the Rh(D) antigen on red blood cells, which is also used in the treatment of patients with ITP. The efficacy of anti-D has been documented in both children and adults with ITP [20–23], even in the clinical setting of HIV-associated ITP [24,25]. The primary mechanism of action of anti-D

Contract grant sponsor: NABI, Rockville, MD; Contract grant sponsor: National Institutes of Health; Contract grant number: 5-T32-CA09307-19.

*Correspondence to: Sherri A. Zimmerman, MD, Box 2916, Duke University Medical Center, Durham, NC 27710. E-mail: Zimme008@mc.duke.edu

Received for publication 3 June 1997; Accepted 27 August 1997

is presumably antibody coating of autologous D positive erythrocytes, leading to RES blockade [20–22,24]. As anti-D, similar to IVIG, may have additional effects on the immune system, we analyzed the in vitro and in vivo immunologic effects of anti-D in children with chronic ITP.

METHODS

In Vitro Evaluation

Venous blood was collected in heparin from pediatric patients with chronic ITP and normal controls. Peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation using a Ficoll-Hypaque gradient. PBMC were resuspended in RPMI 1640 (Gibco, Grand Island, NY) with 10% human A serum (HAS; Worldwide Biologicals, Cincinnati, OH) at 1×10^6/ml and plated in triplicate at 10^5 cells/well in 96-well U-bottom plates (Costar, Cambridge, MA) in the presence of various proliferative stimuli. Final concentrations of stimuli included 2 μg/ml phytohemagglutinin (PHA; Murex Diagnostics, Dartford, England), 20 μg/ml concanavalin A (ConA; Sigma, St. Louis, MO), 2 λ/ml pokeweed mitogen (PWM; GIBCO, Grand Island, NY), 2 λ/well tetanus toxoid antigen (Connaught Laboratory, Swiftwater, PA), and 100 U/ml interleukin-2 (rIL-2; gift from Chiron Corporation, Emeryville, CA). Additives included 10 mg/ml IVIG (Gammimune N 5%; Miles Inc., Elkhart, IN), which is the expected plasma concentration following a standard IVIG infusion of 1 g/kg. Human serum albumin (HSA; Miles Inc.) was used as a control protein at 10 mg/ml. Anti-D (WinRho SD, Rh Pharmaceuticals Inc., Winnipeg, Canada) was used at 0.5 μg/ml, which is the expected plasma concentration following a 50 μg/kg infusion of anti-D, with or without D positive erythrocytes. IVIG was added at 50 μg/ml as a control for the amount of non-specific IgG found in the anti-D preparation. Other controls included RPMI containing 10% human A serum as a negative control and 6 μg/ml dexamethasone (DXM; Sigma) as a positive control. Cells were incubated at 37°C, humidified with 5% CO_2 (4 days for PHA, Con A, and PWM, and 7 days for tetanus and IL-2) and then pulsed with [^{3}H]-thymidine (New England Nuclear, Boston, MA), harvested, and counted on a β-scintillation counter.

In Vivo Evaluation

Patients. Children with chronic ITP and platelets < 30×10^9/L were eligible for anti-D administration if they were D positive, had not undergone splenectomy, and had a hemoglobin concentration >8.0 gm/dl. After written informed consent was obtained using a Duke University IRB-approved protocol, six patients received a single 50 μg/kg IV infusion of anti-D (WinRho-SD) over 5 min, and were studied on day 0 (pre-infusion), day 7, and 1 month later.

Lymphocyte enumeration. Directly labeled monoclonal antibodies (mAbs) conjugated to either fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were added to whole blood as previously described [26]. Specific mAbs included CD3-FITC, CD3-PE, CD4-PE, CD8-FITC, CD8-PE, CD19-PE, CD45-FITC, and CD45RO-PE (Dako Corporation, Carpinteria, CA). Additional mAbs CD45RA-PE and CD56-PE were gifts from Meryl Forman, Coulter Corporation, Hialeah, FL. Analysis of the T cell receptor (TCR) complex included Pan-TCRαβ, TCRγδ, Vα2, Vβ3, Vβ5a, Vβ5b, Vβ5c, Vβ6, Vβ8, Vβ12, Vβ13 (T cell Diagnostics, Cambridge, MA) and Vβ2, Vβ19 (AMAC, Inc., Westbrook, ME), all conjugated to FITC. Control antibodies included IgG-FITC and IgG-PE from Dako. All antibodies were used according to the manufacturers' recommendations. Whole blood (100 μl) was incubated with mAbs for 30 min at 4°C in the dark. Red blood cells were lysed (Ortho Diagnostics, Raritan, NJ) and cells were washed twice, fixed with paraformaldehyde, and analyzed on a FACSCAN (Becton Dickinson, San Jose, CA).

Lymphocyte proliferation assays. PBMC were plated in triplicate in 96-well U-bottom plates at 10^5 cells/well in the presence of mitogens, antigens, or media alone. Final concentrations of stimuli included 2 μg/ml PHA, 20 μg/ml ConA, and 1 λ/ml PWM as 4 day stimuli and 2 λ/well tetanus, and 100 U/ml IL-2 as 7 day stimuli. Cells were incubated at 37°C with 5% CO_2, then pulsed with [^{3}H]-thymidine, harvested, and counted using a β-scintillation counter.

PWM-induced IgG synthesis. PBMC (6×10^6 cells) were cultured in RPMI with 10% HAS in the presence of PWM (1 λ/ml) or media alone in 24-well plates (Costar). After a 7-day incubation in 5% CO_2, supernatant was harvested and stored at −70°C. Total IgG was measured by nephelometry.

Reverse transcriptase-polymerase chain reaction (RT-PCR) for cytokine mRNA levels. PBMC were lysed in guanidine isothiocyanate buffer and total RNA was isolated using a cesium chloride centrifugation method and ethanol precipitation as previously described [27]. Each reverse transcriptase (RT) reaction was performed using random primers and 1.0 μg RNA. One hundred nanograms equivalent of cDNA was amplified by PCR using cytokine-specific primers and a 3-min 94°C denaturation followed by 25 cycles of: 95°C for 1 min, 58°C for 1 min, and 72°C for 1 min as described [28]. Products were labeled with [^{32}P]-dCTP, resolved on a 6% polyacrylamide gel, and exposed to radiographic film (X-omat, Eastman Kodak Co, Rochester, NY) for 1–48 h.

Statistical analysis. Statistical tests were performed using the Primer of Biostatistics (McGraw-Hill, New

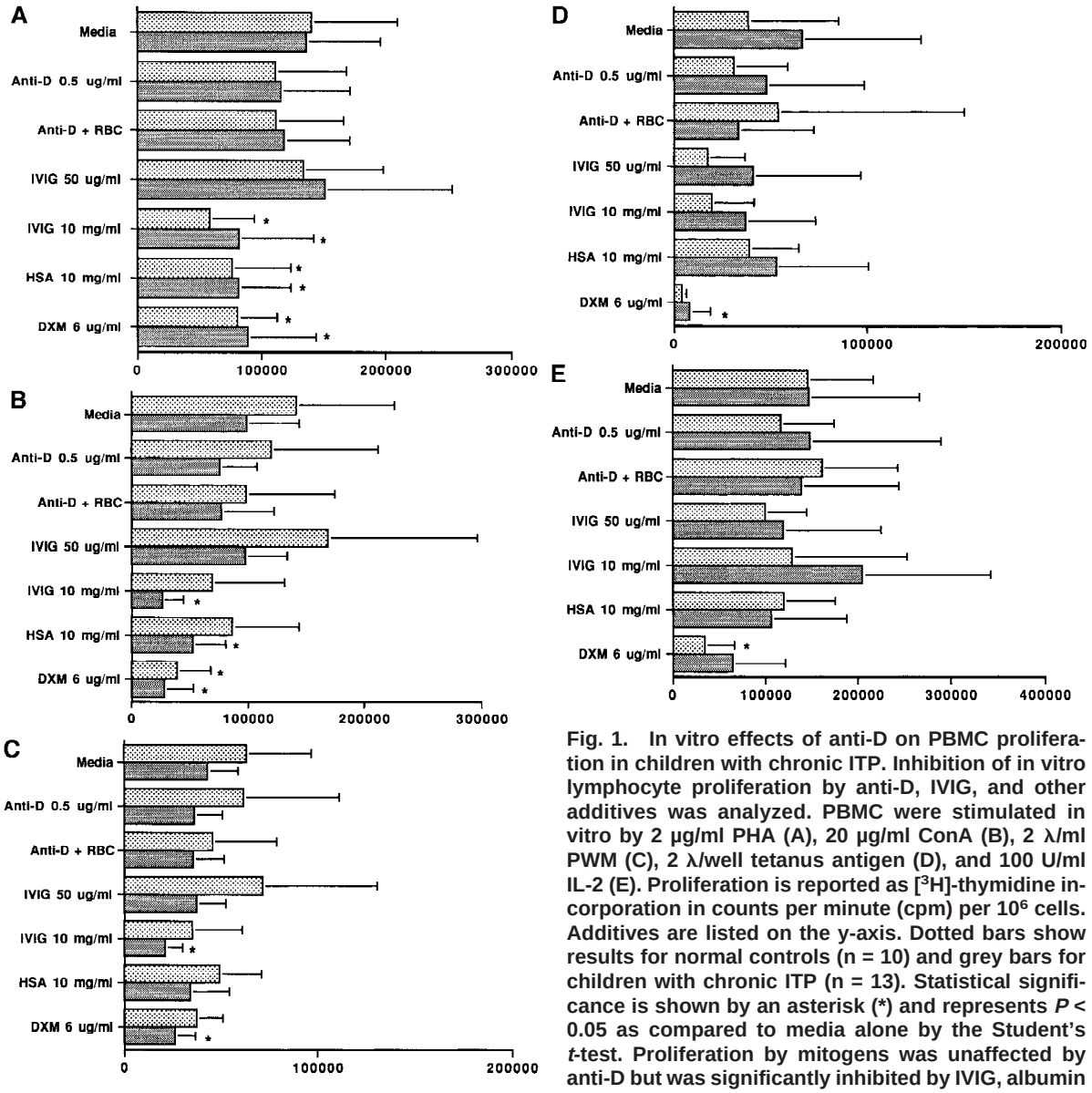

Fig. 1. In vitro effects of anti-D on PBMC proliferation in children with chronic ITP. Inhibition of in vitro lymphocyte proliferation by anti-D, IVIG, and other additives was analyzed. PBMC were stimulated in vitro by 2 μg/ml PHA (A), 20 μg/ml ConA (B), 2 λ/ml PWM (C), 2 λ/well tetanus antigen (D), and 100 U/ml IL-2 (E). Proliferation is reported as [^{3}H]-thymidine incorporation in counts per minute (cpm) per 10^6 cells. Additives are listed on the y-axis. Dotted bars show results for normal controls (n = 10) and grey bars for children with chronic ITP (n = 13). Statistical significance is shown by an asterisk (*) and represents $P < 0.05$ as compared to media alone by the Student's *t*-test. Proliferation by mitogens was unaffected by anti-D but was significantly inhibited by IVIG, albumin (control protein), and dexamethasone (DXM). Proliferation by tetanus or IL-2 was unaffected by anti-D or IVIG, but was significantly inhibited by DXM.

York, NY) software package. The Student's *t*-test was used to compare values between groups.

RESULTS

In Vitro Effects of Anti-D

For the in vitro analysis of the effects of anti-D, T lymphocyte proliferation assays were performed with PBMC from 13 children with chronic ITP and 10 normal controls. Lymphocyte proliferation in the presence of various stimuli and additives is illustrated in Figure 1. In vitro proliferation in response to PHA stimulation (Fig. 1A) was not significantly different between patients and controls. The in vitro addition of IVIG, HSA, and DXM significantly inhibited PHA-induced PBMC proliferation ($P < 0.05$ for each additive), while the addition of anti-D, even in the presence of D positive erythrocytes, had no significant effect on proliferation. Similar results were seen in response to ConA (Fig. 1B). Inhibition of PWM-induced PBMC proliferation (Fig. 1C) was seen with the addition of IVIG and DXM, but not with HSA or anti-D.

In vitro proliferative responses were slightly different in response to antigenic stimuli. As expected with a specific antigen such as tetanus, more variability in individual response was seen than with non-specific mitogenic stimulation. Neither IVIG nor anti-D had any effect on tetanus-induced PBMC proliferation; only the addition of DXM significantly inhibited tetanus-induced

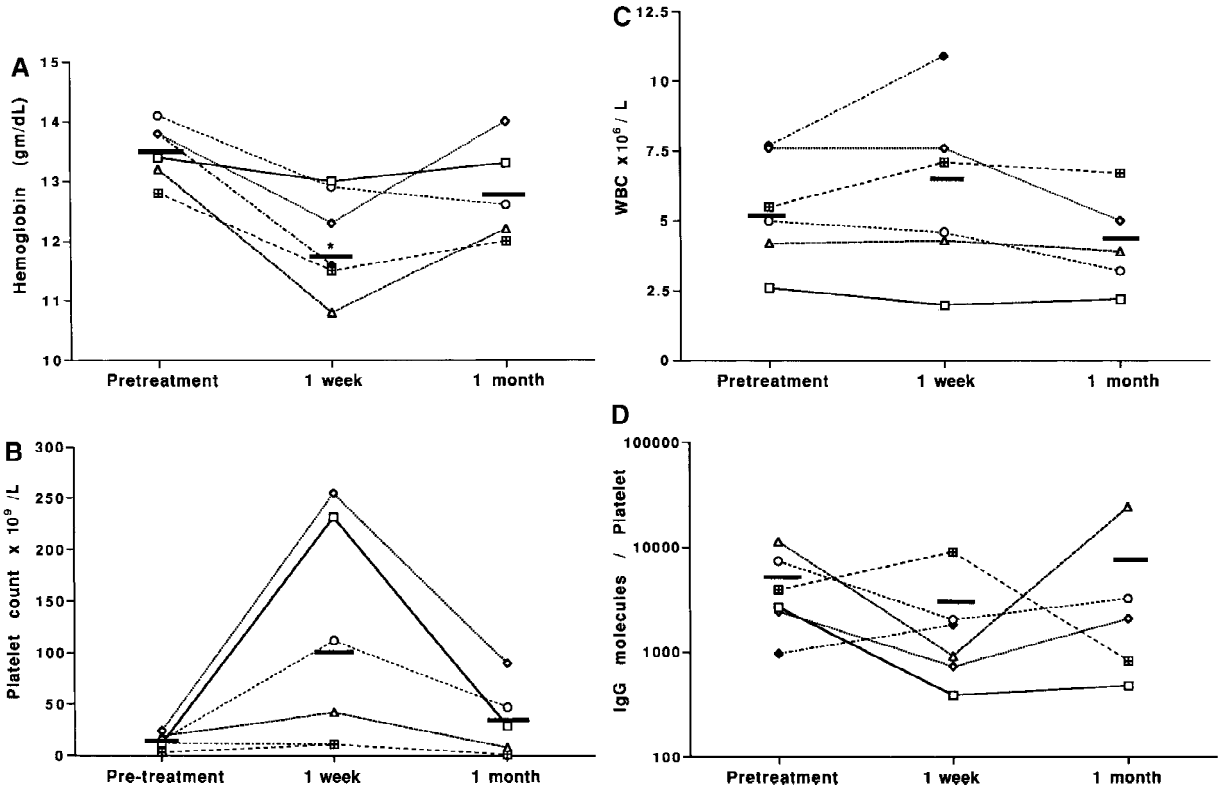

Fig. 2. In vivo effects of anti-D on hematologic values in children with chronic ITP. Clinical laboratory data are illustrated for 6 children with chronic ITP treated with a single IV infusion (50 μg/kg) of anti-D. Laboratory results were obtained at day 0 (pre-treatment), 1 week and 1 month follow-up. The results are shown for hemoglobin (A), platelet count (B), white blood cell count (C), and direct platelet antibodies (D). Each line represents the results for a given patient, and the horizontal bars represent the mean value. For one patient who withdrew from the study, no results were available for the 1 month follow-up. Statistical significance is shown by an asterisk (*) and represents $P < 0.05$ by the Student's *t*-test. Only the hemoglobin concentration showed a significant change between day 0 and 1 week (mean change 1.5 ± 0.7 gm/dL, $P = 0.005$).

PBMC proliferation (Fig. 1D). Similarly IL-2 induced PBMC proliferation (Fig. 1E) was significantly inhibited only by DXM. Neither IVIG, HSA, nor anti-D affected proliferation to these stimuli.

In Vivo Effects of Anti-D

Patients. A total of 6 children with chronic ITP had immunologic evaluations performed before and after administration of IV anti-D. The study group included 4 males and 2 females (5 white, 1 black) with a mean age of 15.8 ± 1.1 years. All patients had received prior therapy with either corticosteroids, IVIG, or both.

Clinical laboratory data. Laboratory results for the 6 children with chronic ITP treated with a single IV infusion of anti-D are summarized in Figure 2. There was a significant drop in the hemoglobin (Fig. 2A, 1.5 ± 0.7 gm/dl, $P = 0.005$) between day 0 (pre-treatment) and 1 week. None of the children was symptomatic from anemia and most had recovery towards their baseline value by 1 month. Three children had a good response in their platelet count (Fig. 2B), one child had a modest increase, and two had no response at all. The mean increase in platelet count between day 0 and day 7 was 96 ± 106 × 10^9/L ($P = 0.057$), but did not achieve statistical significance because of the wide range of response. There was no significant change in white blood cell count (Fig. 2C) or direct platelet antibody response (Fig. 2D) following anti-D infusion. When responders and nonresponders were analyzed separately, however, 3 of the 4 responders had a marked decrease in the level of antibody detected on the platelet surface.

Lymphocyte enumeration. Table I lists the results (mean ± standard deviation) for lymphocyte immunophenotypic analysis performed prior to, 1 week, and 1 month following the single IV infusion of anti-D. There were no significant changes in lymphocyte subsets including T cells (CD3), T suppressor cells (CD8), T helper cells (CD4), B cells (CD19), natural killer (NK) cells (CD56), TCRαβ or TCRγδ cells, naive (CD45RA/CD3) T cells, or memory (CD45RO/CD3) T cells, the CD4/CD8 ratio, or the TCR variable beta repertoire.

Lymphocyte proliferation responses. Following the anti-D infusion, PBMC proliferative responses to a variety of in vitro stimuli were measured (Fig. 3). Prolifera-

TABLE I. In Vivo Immunologic Effects of Anti-D on Lymphocyte Enumeration in Children With Chronic ITP*

	Day 0	Day 7	Day 28
CD3	71.9 ± 6.0	67.0 ± 6.8	65.3 ± 13.0
CD4	41.9 ± 7.9	39.0 ± 5.1	32.8 ± 9.7
CD8	21.0 ± 6.7	18.8 ± 4.0	15.9 ± 6.4
CD4/CD8	2.2 ± 1.0	2.2 ± 0.6	2.4 ± 1.1
CD19	13.2 ± 4.5	12.2 ± 7.3	10.1 ± 3.1
CD45	94.4 ± 3.2	92.1 ± 7.8	90.3 ± 4.7
CD56	9.4 ± 3.2	8.2 ± 3.9	10.0 ± 3.0
CD45RA/CD3	54.7 ± 16.0	53.5 ± 5.5	49.8 ± 23.3
CD45RO/CD3	35.5 ± 12.5	32.5 ± 13.9	27.8 ± 9.6
TCRαβ/CD3	93.4 ± 3.2	95.8 ± 2.3	92.6 ± 2.9
TCRγδ/CD3	4.5 ± 3.6	3.0 ± 1.9	6.0 ± 3.2
Vα2	2.1 ± 0.4	2.4 ± 0.7	2.2 ± 0.9
Vβ2	0.1 ± 0.1	0.4 ± 0.2	0.1 ± 0.02
Vβ3	1.9 ± 1.3	1.9 ± 1.7	1.7 ± 1.4
Vβ5a	1.3 ± 0.6	1.1 ± 0.8	1.0 ± 0.7
Vβ5b	0.6 ± 0.2	0.5 ± 0.3	0.6 ± 0.4
Vβ5c	1.7 ± 0.8	1.9 ± 0.6	1.8 ± 0.7
Vβ6	2.1 ± 1.1	2.0 ± 1.3	1.7 ± 0.8
Vβ8	3.8 ± 0.9	5.0 ± 1.7	4.3 ± 1.4
Vβ12	1.2 ± 0.3	1.4 ± 0.3	1.2 ± 0.3
Vβ13	1.1 ± 0.4	1.3 ± 0.1	1.1 ± 0.2
Vβ19	2.9 ± 1.7	2.7 ± 1.9	2.4 ± 1.7

*Results are listed as the mean percentage of cells (mean ± 1 standard deviation). None of the lymphocyte subsets was significantly different between day 0, day 7, and day 28.

tive responses to mitogens including PHA (Fig. 3A), ConA (Fig. 3B), and PWM (Fig. 3C) demonstrated a trend towards increased proliferation at day 7 following infusion of anti-D, but no significant changes were found. Proliferative responses to tetanus toxoid antigen (Fig. 3D) and IL-2 (Fig. 3E) were not significantly changed following anti-D treatment.

PWM-induced IgG biosynthesis. To assess the effects of anti-D infusion on B cell function and polyclonal immunoglobulin synthesis, in vitro IgG production in response to PWM was examined. Results are shown in Figure 4 and illustrate no change in the mean IgG level following anti-D infusion. Specifically, no in vitro suppression of IgG synthesis was seen following a single IV infusion of anti-D.

Cytokine mRNA levels. RT-PCR analysis of cytokine mRNA production was performed to assess the effects of anti-D on PBMC cytokine production. Figure 5 shows results for IL-4, IL-10, IFN-γ, and β-actin mRNA levels following the anti-D infusion. No differences in cytokine mRNA levels were seen between day 0, day 7, and day 28. Similar analysis was done for IL-2, TNF-α, TNF-β mRNA levels, and no changes were detected (not shown).

DISCUSSION

Both IVIG and anti-D are commonly used for the treatment of ITP in children and adults. In addition to its use for patients with ITP, however, IVIG has also been used in many other clinical settings including primary immunodeficiency disorders [29], neonatal sepsis [30], Kawasaki disease [31], and in neurological disorders such as myasthenia gravis, Guillain-Barre syndrome, or intractable seizures [32]. In each of these instances, IVIG is used either to provide passive antibody transmission or to modify the immune response in a host with presumed autoimmune disease. The ability of IVIG to modulate the immune system has been extensively studied, yet the success of IVIG in the treatment of these various clinical disorders is not well understood [33]. Since anti-D is also an effective treatment in ITP, we evaluated the in vitro and in vivo immunomodulatory effects of anti-D in children with chronic ITP.

Comparison of In Vitro Effects of Anti-D and IVIG

Our analysis of in vitro T lymphocyte proliferation assays in children with chronic ITP revealed that anti-D at 0.5 μg/ml caused no inhibition of in vitro T lymphocyte proliferation in response to PHA, ConA, or PWM, either alone or in the the presence of D positive erythrocytes (Fig. 1). Similarly, IVIG at 50 μg/ml, equivalent to the amount of non-specific IgG found in anti-D, did not affect in vitro T lymphocyte proliferation. Dexamethasone, however, significantly inhibited in vitro T lymphocyte proliferation in response to PHA, ConA, PWM, tetanus toxoid antigen, and IL-2.

We found that IVIG at 10 mg/ml significantly inhibited T lymphocyte proliferation induced by PHA, ConA, and PWM (Fig. 1). These results are similar to those reported previously where dose dependent in vitro inhibition of T lymphocyte proliferation was demonstrated [14–16]. We found, however, that non-specific protein (10 mg/ml human serum albumin) was equally suppressive as IVIG in in vitro T lymphocyte proliferation assays. This finding was surprising since previous reports by van Schaik et al. and Klaesson et al. found no inhibition by HSA when added at concentrations similar to those used for IVIG [15,16]. Our results suggest that at least part of the effect of IVIG may be related to non-specific effects from high concentrations of protein, and not to specific anti-idiotype antibodies or Fc receptor blockade.

In Vivo Effects of Anti-D

We then performed an analysis of the in vivo immunomodulatory changes associated with a single IV infusion of anti-D in six children with chronic ITP. Similar to the results reported by Bussel et al. and Scaradavou et al. [24,25], we found an increase in platelet count of $>20 \times 10^9$/L in most children following a single IV infusion of anti-D (WinRho-SD, 50 μg/kg). The mean increase in platelet count was $96 \pm 106 \times 10^9$/L, but did not achieve statistical significance because of the wide range of re-

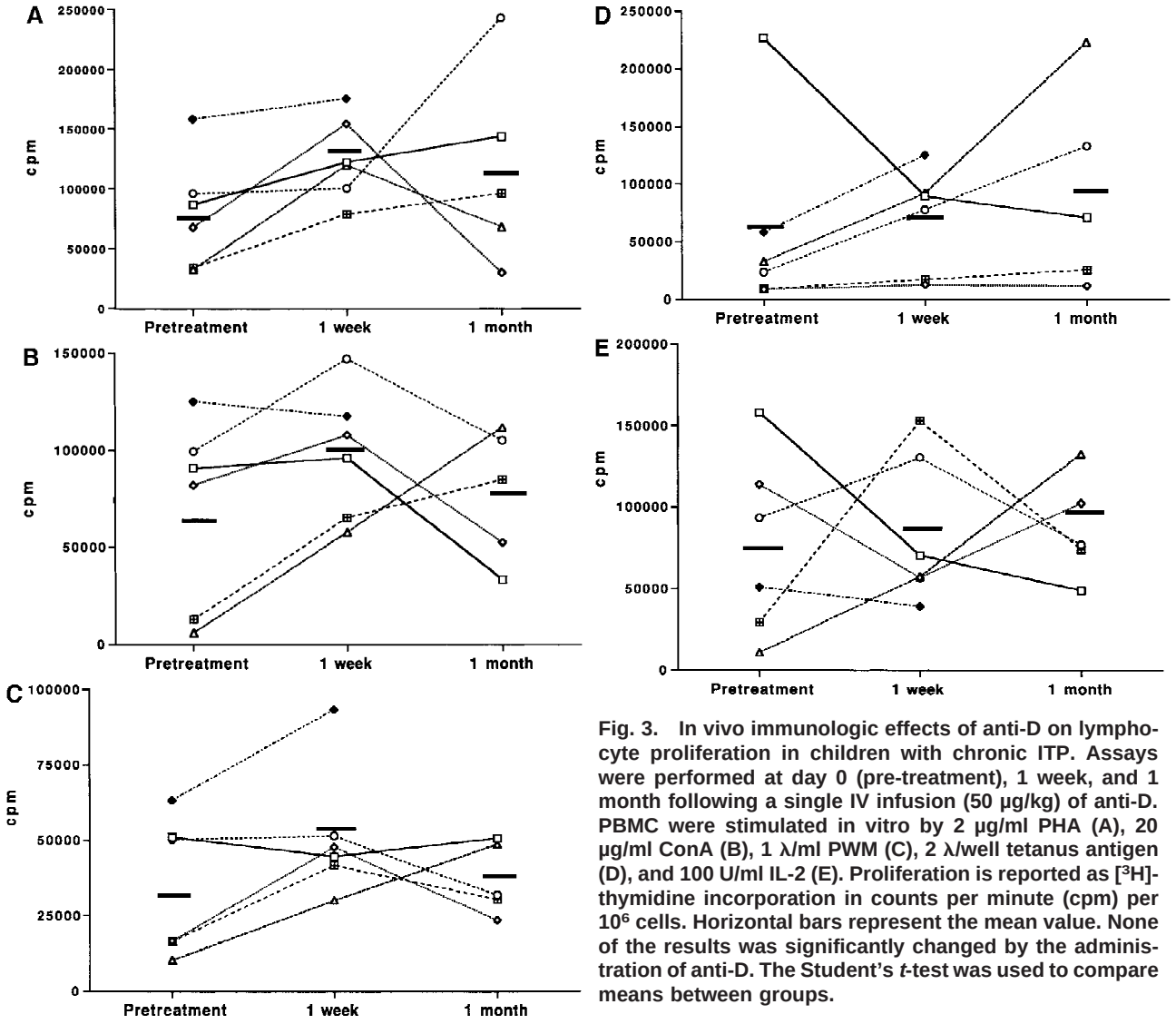

Fig. 3. In vivo immunologic effects of anti-D on lymphocyte proliferation in children with chronic ITP. Assays were performed at day 0 (pre-treatment), 1 week, and 1 month following a single IV infusion (50 µg/kg) of anti-D. PBMC were stimulated in vitro by 2 µg/ml PHA (A), 20 µg/ml ConA (B), 1 λ/ml PWM (C), 2 λ/well tetanus antigen (D), and 100 U/ml IL-2 (E). Proliferation is reported as [^{3}H]-thymidine incorporation in counts per minute (cpm) per 10^6 cells. Horizontal bars represent the mean value. None of the results was significantly changed by the administration of anti-D. The Student's *t*-test was used to compare means between groups.

sponse (Fig. 2B). There was, however, a significant decrease in hemoglobin of 1.5 ± 0.7 gm/dl, but no changes in white blood cell count, again similar to that previously reported [24,25]. The decrease in direct platelet antibody levels observed in responders could reflect true suppression of autoantibody synthesis. Alternatively, the observation of a reduction in the average number of IgG molecules per platelet may simply result from an increase in platelet count. None of our patients experienced significant toxicity from the anti-D infusion.

We first evaluated in vitro proliferative responses to PHA, ConA, PWM, tetanus toxoid antigen, and IL-2 in children with chronic ITP following the single infusion of anti-D. Anti-D had no significant effect on in vitro T lymphocyte proliferation in response to mitogens or antigens (Fig. 3). These results contrast the reported in vivo immunomodulatory effects of IVIG administration, which include suppression of in vitro T lymphocyte PHA, ConA, PWM, IL-2, and mixed lymphocyte culture (MLC) proliferative responses [14–16]. IVIG treatment in adults with ITP also results in reduced response to PWM and *Staphylococcus aureus* toxin [13].

We next performed lymphocyte enumeration studies following a single IV infusion of anti-D (Table I) and found no significant changes in lymphocyte subsets, CD4/CD8 ratio, or TCR variable beta repertoire. IVIG has been shown to affect lymphocyte subsets although the results have been conflicting. A relative increase of suppressor T cells with a decrease in the CD4/CD8 ratio has been reported in adults with ITP following IVIG therapy [11–13,18]. Unlike IVIG, anti-D does not appear to affect lymphocyte subsets.

In addition to its effects on T lymphocytes, in vivo IVIG administration has been shown to affect B-cell function, both by effects on platelet associated IgG and by suppression of in vitro PWM-induced immunoglobu-

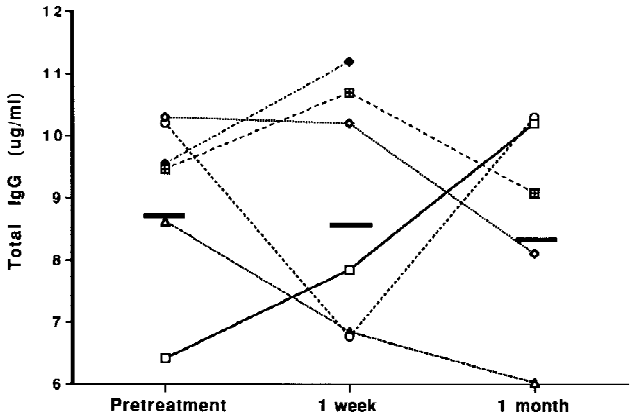

Fig. 4. Effects of anti-D on in vitro PWM-induced IgG synthesis in children with chronic ITP. Evaluations were performed at day 0, 1 week, and 1 month following administration of anti-D. IgG was measured by nephelometry. Mean values for each time point are illustrated as a solid bar. No significant difference in IgG synthesis was noted between day 0, day 7, and day 28 values.

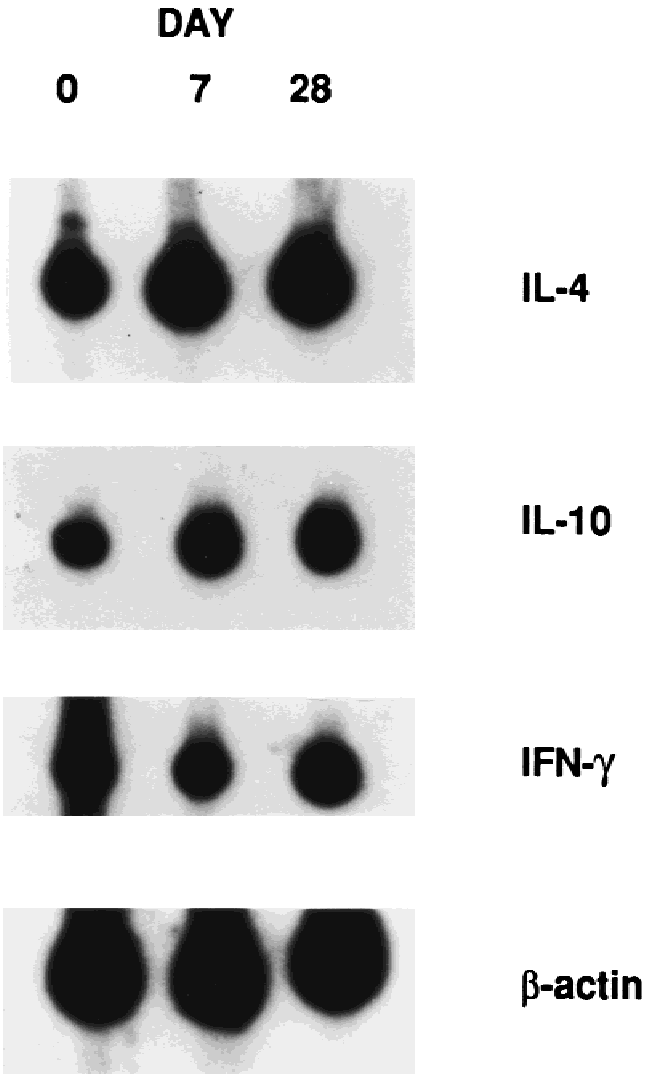

Fig. 5. In vivo immunologic effects of anti-D on PBMC cytokine mRNA synthesis in children with chronic ITP. RT-PCR with cytokine-specific primers was performed at day 0, 1 week, and 1 month following a single dose of anti-D. No change in mRNA levels was noted for cytokines IL-4, IL-10, or IFN-γ. β-actin was used as a control.

lin production. Antiplatelet antibody titers have variously been reported to increase [34] or decrease [12,18,19,34] following treatment with IVIG. Other effects on B lymphocytes include suppression of PWM-induced B cell differentiation [17] and reduced in vitro PWM-induced immunoglobulin biosynthesis, IgG more than IgA or IgM [11,12]. Bussel et al. [19], however, reported increased in vitro immunoglobulin synthesis following IVIG therapy. In our group of children with chronic ITP, a single IV infusion of anti-D had no significant effect on in vitro PWM-induced IgG biosynthesis (Fig. 4).

Given the complex interactions between T and B lymphocytes, much of which is mediated by cytokines, IVIG and anti-D could also affect immune regulation by effects on cytokine production. We examined cytokine mRNA production by RT-PCR and found no differences in IL-2, IL-4, IL-10, TNF-α, TNF-β or IFN-γ mRNA levels following a single IV infusion of anti-D (Fig. 5). These cytokines were analyzed because of their central roles in T lymphocyte growth, regulation, and differentiation. Our results contrast those by Andersson et al. [35], who found that IVIG significantly down-regulated production of IL-2, IL-10, IFN-γ, and TNF-β, but had modest or no effect on IL-8 and TNF-α protein levels.

IVIG has been shown to inhibit in vivo clearance of autologous IgG-sensitized erythrocytes, presumably by Fc receptor blockade as well as decreasing the affinity of Fc receptor binding for IgG-coated platelets [5,10]. Anti-D leads to similar RES blockade in ITP by preferential destruction of antibody sensitized erythrocytes over antibody-coated platelets [20–22]. This RES blockade is relatively short-lived, however, returning to baseline by approximately 1 month after therapy, suggesting some other mechanism might be involved to cause more prolonged responses to IVIG or anti-D [5]. In our studies, we did not find any evidence that anti-D has significant in vitro or in vivo immunomodulatory or immunosuppressive effects, suggesting it probably functions primarily by RES blockade in children with ITP.

ACKNOWLEDGMENTS

The authors thank Dr. Kathy M. Zunich for her support of this project. This work was supported by a grant from NABI, Rockville, MD and by grant 5-T32-CA09307-19 from the National Institutes of Health.

REFERENCES

1. Ware R, Kinney TR: Immunopathology of childhood idiopathic thrombocytopenic purpura. CRC Crit Rev Oncol/Hematol 7:169, 1987.
2. Ware R, Kinney TR: Therapeutic considerations in childhood idiopathic thrombocytopenic purpura. CRC Crit Rev Oncol/Hematol 7: 139, 1987.
3. Imbach P, Barandun S, Baumgartner C, Hirt A, Hofer F, Wagner HP: High-dose intravenous gammaglobulin therapy of refractory, in particular idiopathic thrombocytopenia in childhood. Helv Paediatr Acta 36:81, 1981.
4. Imbach P, Barandun S, d'Apuzzo V, Baumgartner C, Hirt A, Morell A, Rossi E, Schoni M, Vest M, Wagner HP: High-dose/intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. Lancet 1:1228, 1981.
5. Fehr J, Hofmann V, Kappeler CM: Transient reversal of thrombocytopenia in idiopathic thrombocytopenic purpura by high-dose intravenous gamma globulin. N Engl J Med 306:1254, 1982.
6. Bussel JB, Schulman I, Hilgartner MW, Barandun S: Intravenous use of gammaglobulin in the treatment of chronic immune thrombocytopenic purpura as a means to defer splenectomy. J Pediatr 103:651, 1983.
7. Bussel JB, Goldman A, Imbach P, Schulman I, Hilgartner MW: Treatment of acute idiopathic thrombocytopenia of childhood with intravenous infusions of gammaglobulin. J Pediatr 106:886, 1985.
8. Bussel JB, Pham LC, Aledort L, Nachman R: Maintenance treatment of adults with chronic refractory immune thrombocytopenic purpura using repeated intravenous infusions of gammaglobulin. Blood 72: 121, 1988.
9. Kurlander R: Reversible and irreversible loss of Fc receptor functions of human monocytes as a consequence of interaction with immunoglobulin G. J Clin Invest 66:773, 1980.
10. Kimberly RP, Salmon JE, Bussel JB, Crow MK, Hilgartner MW: Modulation of mononuclear phagocyte function by intravenous γ-globulin. J Immunol 132:745, 1984.
11. Tsubakio T, Kurata Y, Katagiri S, Kanakura Y, Tamaki T, Kuyama J, Kanayama Y, Yonezawa T, Tarui S: Alteration of T cell subsets and immunoglobulin synthesis in vitro during high dose γ-globulin therapy in patients with idiopathic thrombocytopenic purpura. Clin Exp Immunol 53:697, 1983.
12. Dammacco F, Iodice G, Camptbasso N: Treatment of adult patients with idiopathic thrombocytopenic purpura with intravenous immunoglobulin: Effects on circulating T cell subsets and PWM-induced antibody synthesis in vitro. Br J Haematol 62:125, 1986.
13. Macey MG, Newland AC: CD4 and CD8 subpopulation changes during high dose intravenous immunoglobulin treatment. Br J Haematol 76:513, 1990.
14. Kawada K, Terasaki PI: Evidence for immunosuppression by high-dose gammaglobulin. Exp Hematol 15:133, 1987.
15. Klaesson S, Ringden O, Markling L, Remberger M, Lundkvist I: Immune modulatory effects of immunoglobulins on cell-mediated immune responses in vitro. Scand J Immunol 38:477, 1993.
16. Van Schaik IN, Lundkvist I, Vermeulen M, Brand A: Polyvalent immunoglobulin for intravenous use interferes with cell proliferation in vitro. J Clin Immunol 12:325, 1992.
17. Stohl W: Cellular mechanisms in the in vitro inhibition of pokeweed mitogen-induced B cell differentiation by immunoglobulin for intravenous use. J Immunol 136:4407, 1986.
18. Delfraissy JF, Tchernia G, Laurian Y, Wallon C, Galanaud P, Dormont J: Suppressor cell function after intravenous gammaglobulin treatment in adult chronic idiopathic thrombocytopenic purpura. Br J Haematol 60:315, 1985.
19. Bussel J, Pahwa S, Porges A, Cunningham-Rundles S, Koziner B, Morell A, Barandun S: Correlation of in vitro antibody synthesis with the outcome of intravenous γ-globulin treatment of chronic idiopathic thrombocytopenic purpura. J Clin Immunol 6:50, 1986.
20. Salama A, Kiefel V, Amberg R, Mueller-Eckhardt C: Treatment of autoimmune thrombocytopenic purpura with rhesus antibodies (anti-Rh_o(D)). Blut 49:29, 1984.
21. Salama A, Kiefel V, Mueller-Eckhardt C: Effect of IgG anti-Rh_o(D) in adult patients with chronic autoimmune thrombocytopenia. Am J Hematol 22:241, 1986.
22. Becker T, Kuenzlen E, Salama A, Mertens R, Kiefel V, Weiß H, Lampert F, Gaedicke G, Mueller-Eckhardt C: Treatment of childhood idiopathic thrombocytopenic purpura with rhesus antibodies (anti-D). Eur J Pediatr 145:166, 1986.
23. Andrew M, Blanchette VS, Adams M, Ali K, Barnard D, Chan KW, DeVeber LB, Esseltine D, Israels S, Korbrinsky N, Luke B, Milner RA, Woloski MR, Vegh P: A multicenter study of the treatment of childhood chronic idiopathic thrombocytopenic purpura with anti-D. J Pediatr 120:522, 1992.
24. Bussel JB, Graziano JN, Kimberly RP, Pahwa S, Aledort LM: Intravenous anti-D treatment of immune thrombocytopenic purpura: Analysis of efficacy, toxicity, and mechanism of effect. Blood 77: 1884, 1991.
25. Scaradavou A, Woo B, Woloski BMR, Cunningham-Rundles S, Ettinger LJ, Aledort LM, Bussel JB: Intravenous anti-D treatment of ITP: Experience in 272 patients. Blood 89:2689, 1997.
26. Ware RE, Howard TA: Elevated numbers of gamma-delta (γδ+) T lymphocytes in children with immune thrombocytopenic purpura. J Clin Immunol 14:237, 1994.
27. Ware RE, Hart MK, Haynes BF: Induction of T cell CD7 gene transcription by nonmitogenic ionomycin-induced transmembrane calcium flux. J Immunol 147:2787, 1991.
28. Winter SS, Howard TA, Ritchey AK, Keller FG, Ware RE: Elevated levels of tumor necrosis factor-beta, gamma-interferon, and IL-6 mRNA in Castleman's disease. Med Pediatr Oncol 26:48, 1996.
29. Rosenblatt HM: Primary immunodeficiency disorders and the rational use of intravenous immunoglobulin. Semin Pediatr Infect Dis 3:150, 1992.
30. Boom JA, Feigin RD: The use of intravenous immunoglobulin in the prevention and treatment of neonatal sepsis. Semin Pediatr Infect Dis 3:167, 1992.
31. Fong KJ, Kaplan SL: The use of intravenous immunoglobulin in Kawasaki disease. Semin Pediatr Infect Dis 3:174, 1992.
32. Lee RT, Lo LL: The use of intravenous immunoglobulin in neurological diseases. Semin Pediatr Infect Dis 3:191, 1992.
33. Abramson SL: Nontraditional uses of intravenous immunoglobulin and directions for future research. Semin Pediatr Infect Dis 3:199, 1992.
34. Kurata Y, Tsubakio T, Yonezawa T, Tarui S: High-dose gammaglobulin therapy for idiopathic thrombocytopenic purpura in adults. Acta Haematol 69:391, 1983.
35. Andersson UG, Bjork L, Skansen-Saphir U, Andersson JP: Down-regulation of cytokine production and interleukin-2 receptor expression by pooled human IgG. Immunology 79:211, 1993.